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# MICROBIOLOGICAL EVALUATION OF ICE CREAM MIX POWDER IN ASSIUT CITY

(With 9 Tables & 2 Figures)

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## التقييم الميكروبيولوجي لبودرة مخلوط الآيس كريم بمدينة أسيوط

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اجرى الفحص الميكروبيولوجى لمائة عينه عشوائيه من بودرة مخلوط الآيس كريم المطعمه بالشيكولاته، المانجو، الفراوله، الفانيليا ( ٢٥ عينه من كل نوع) والتى جمعت على مدار العام من محلات البقاله والسوبر ماركت فى مدينة أسيوط وذلك لمعرفة الحاله الصحيه ومدى تلوثها بالميكروبات المختلفه. وقد تبين من الفحص ان متوسط اعداد *الميكروبات الكليه، البكتريا المحبه للبروده، الباسيللس سيريس، البكتريا المعويه، البكتريا القولونيه، البكتريا القولونيه البرازيه، الانتيروكوكاى*، الفطريات في تلك العينات كان كالتالى : ١ x ١٠^٧، ٨ x ١٠^٤، ٣ x ١٠^٤، ٥ x ١٠، ٥، ٤، ٢ x ١٠^٣، ٩ x ١٠^٢ ميكروب/ جرام على الترتيب. أما النسبه المئويه لتواجد تلك الميكروبات سالفة الذكر فكانت على النحو التالى: ١٠٠، ١٠٠، ٧٩، ٤٢، ٩، ٢، ٢٣، ١٠٠٪ على التوالى لعينات مخلوط الآيس كريم الجاف المطعم بالاضافات المختلفه. أما نسبة وجود ال *Staph. epidermidis* وال *Micrococci* وكذلك ال *Clostridia* فى داخل العينات المفحوصه كانت تتراوح ما بين ٨ الى ٣٢٪، صفر الى ١٦٪، ٢٨ الى ٥٦٪ على الترتيب. بينما لم يتم عزل ميكروبى *الايشيريشياكولاى والمكور العنقودى الذهبى* من جميع العينات السابقه. وفى النهايه القى الضوء على الأهميه الصحيه والاقتصاديه التى تترتب على استهلاك تلك المنتجات الملوثه بالميكروبات المختلفه. كما ناقش البحث الاحتياطات الواجب اتخاذها لمنع التلوث بدءاً من أول مراحل الانتاج ومنتهياً بالوصول الى المستهلك، كى تصل بودرة مخلوط الآيس كريم المطعمه بالنكهات المتباينه الى حيز الوجود بصورة صحيه سليمه وجودة عالية.

## SUMMARY

A hundred random samples of ice cream mix powder flavoured with chocolate, mango, strawberry and vanilla (twenty five of each), were collected from Assiut City markets and groceries over a period of one year to determine their microbiological quality. The average counts of *aerobic plate, psychrotrophic, B.cereus, enterobacteriaceae, coliforms, fecal coliforms, enterococci* and total yeast and mould in the examined samples were $1x10^7$, $8x10^4$, $3x10^4$, 5x10, 5, 4, $2x10^3$ and $9x10^2$ cells/g, respectively. The incidences of *psychrotrophs, B.cereus, enterobacteriaceae, coliforms, fecal coliforms, enterococci* and total yeast & mould were

respectively, 100, 79, 42, 9, 2, 23 and 100 %. On the other hand, Staph. *epidermidis, micrococci* and *clostridia* were present in 21, 8 and 41 %, respectively. While, *E. coli* and *Staph. aureus* failed to be isolated from the examined samples. The public health importance and suggestive measures for improving the quality of ice cream mix powder have been discussed.

***Key words:*** *Ice-cream mix powder-Microbiology-Assiut*

## INTRODUCTION

Nowadays, ice cream mix powders are produced and sold in increasing quantities for preparation of ice cream on a large scale. The manufacture of dried ice cream mix comprises blending a standardized mix of whole milk and cream with sugar, stabilizers, emulsifiers, antioxidants and other additives such as dried egg yolk, dried buttermilk, sodium caseinate and flavouring agents. The processing involves homogenization at preferably 2500 lb/in$^2$ and 150-160$^o$F before drying, pasteurization, aging, spray or roller drying to 1-2 % moisture, cooling rapidly after drying, packaging hermetically and storaged at low temperature and low relative humidity conditions (Favstova, 1971; Arora and Sudarsanam, 1986; Goyal, et al., 1987 and Bhandari and Balachandran, 1988).

In spite of the temperature attained in preparation of ice cream mix powder and its low moisture content, it may, at times, be responsible for transmitting some pathogenic microorganisms to consumers. These organisms may find opportunity, in absence of heat treatment, to multiply during preparation of ice cream, either at home or in small factories (small cabinets using primitive processing equipments), reaching counts sufficient to be of public health hazards.

Presence of *B. cereus, enterobacteriaceae, coliforms, fecal coliforms, E. coli, Staphylococci* and *clostridia* could be dangerous sources of infections and food poisoning outbreaks. On the other hand, *coliforms* and *enterococci* are useful index in determining the sanitary quality of the dairy products. Also, the *enterococci* and *clostridia* have a distinctive role as an indicator of poor factory sanitation, owing to their relatively high resistance to drying. While, *aerobic plate bacteria, psychrotrophs* and *yeasts & moulds* are indicative of poor sanitary practices in preparation, packaging, storage and distribution of such products. Several reports on the microbiological quality of ice cream mix powder have been conducted by Stec and Burzynska (1980); El-Bassiony and Aboul-Khier (1983) and Aboul-Khier, et al. (1985).

Therefore, this work was planned to investigate the microbiological status of ice cream mix powders with various flavouring agents available in Assiut City.

## MATERIALS and METHODS

**Collection and preparation of samples** :

One hundred random samples of ice cream mix powder with chocolate, mango, strawberry and vanilla flavours (25 each) were collected from different groceries and supermarkets in Assiut City over a period of one year (from june 1995 to june 1996). The samples were dispatched directly to the laboratory where they were prepared for microbiological examination according to A.P.H.A., (1985).

**1- *Aerobic plate count:***

The technique adopted is that recommended by A.P.H.A. (1985), using Standard Plate Count agar.

**2- Psychrotrophic count**:

Crystal Violet Tetrazolium agar medium was used for a rapid enumeration of *psychrotrophic* bacteria as described by Gilliland, et al. (1976).

**3- Enumeration and isolation of *Bacillus cereus*** (Goepfert, 1976).

Surface plating technique of each sample and its dilutions onto KG agar plates (Kim and Goepfert, 1971) was used for enumeration of *B. cereus*. The identification of the suspected colonies were carried out according to Kramer, et a'. (1982) and Parry, et a'. (1983).

**4- *Enterobacteriaceae* count**:

Violet Red Bile Glucose agar was used for *enterobacteriaceae* count as performed by Mercuri and Cox (1979).

**5- Total *coliforms* count**:

Coliforms were estimated by a 3-tube Most Probable Number (MPN) technique as recommended by A.O.A.C. (1975).

**6- *Fecal coliforms* count**: (MPN/g)

Numbers of *fecal coliforms* were determined using Escherichia Coli (EC) broth tubes according to A.O.A.C. (1975).

**7- *Escherichia coli* count**: (MPN/g)

Enumeration of *Escherichia coli* were carried out according to A.O.A.C. (1975).

**8- *Enterococci* count**:

KF Streptococcus agar was used for *enterococci* count as performed by Deibel and Hartman (1976).

**9- *Staphylococcus aureus* count**:

Baird-Parker agar plates (Baird-Parker, 1962) were used for enumeration of Staph. aureus.

**10- Isolation and identification of other *staphylococci* and *micrococci*** :

The isolation and identification of *Staph. epidermidis* and *micrococci* were carried out according to Finegold and Martin (1982).

**11- Detection of *anaerobic sporeformers*** : (Stormy Fermentation Test).

The technique adopted is that described by Cruickshank, et al., (1969).

**12- Total *yeast* and *mould* counts**:

Malt Extract agar was used according to Harrigan and Margaret (1976).

## RESULTS

The results were recorded in Tables from 1 to 9 & Figures 1 and 2.

## DISCUSSION

According to the data summarized in Table 1, the min. count of *aerobic plate*/g of the total ice cream mix powder samples was $1x10^3$, while the max. was $1x10^8$ with an average of $1x10^7$. A higher average count was observed in dried ice cream mix with mango ($2x10^7$ bacteria/g), and a lower value was detected in ice cream mix powder with vanilla ($6x10^6$ bacteria/g). Lower results were recorded by El-Bassiony and Aboul-Khier (1983). The higher counts of *aerobic plate* in the examined samples of ice cream mix powder could be attributed to the microbial content of raw materials, the uneffectiveness of processing procedures and the negligent sanitary control of equipment and utensils at the processing plant. One or more of these factors, if not adequately controlled can be responsible for higher than expected *aerobic plate counts* during and after processing (National Academy of Sciences, 1985). Moreover, the *aerobic plate count* is recommended by several investigators as index of sanitary quality, organoleptic quality, safety and utility of foods.

As recorded in Table 2 and Figure 1, all of the examined samples proved to be contaminated with *psychrotrophic* bacteria. The counts ranged from $2x10^2$ to $7x10^5$ with an average of $8x10^4$/g. The highest number was noticed in dried ice cream mix samples with strawberry flavour with an average of $1x10^5$ bacteria/g. The high incidence and counts of *psychrotrophs* detected in the examined samples could be attributed to a history of unsanitary processing, handling and storage. Furthermore, presence of these organisms in ice cream mix powder samples implies existence of heat resistant types such as *Bacillus* and *Clostridium* (Larkin and Stokes, 1966; Bhadsavle et al., 1972 and Baker and Griffiths, 1993).

*B. cereus*, as recorded in Table 3 and Figure 1 was existed in 79 % of ice cream mix powder samples in numbers varied from <100 to $7x10^5$ with an average value of $3x10^4$/g. The majority of positive samples contaminated with *B. cereus* were ice cream mix powder with mango flavour. A lower counts were detected in ice cream mix powder with strawberry flavour (average $6x10^3$/g), while a higher numbers of *B. cereus* were observed in samples with chocolate flavour (average $8x10^4$/g). Stec and Burzynska (1980) could isolate *B. cereus* from samples of dried ice cream mix. The obtained results indicate that ice cream mix powder could be responsible for transmitting *B. cereus* to consumers. However, the contamination by these organisms is usually in the form of heat resistant endospores which survive processing temperatures (Davis and Wilkinson, 1973). On the other hand, Sutherland and Limond (1993) and others stated that the presence of high numbers of *B. cereus* organisms in a variety of foods has been found to be associated with the production of diarrhoeagenic

toxin. Moreover, Baker and Griffiths (1993) mentioned that in Canada, between the years 1975 and 1986, the number of reported cases of foodborne illness caused by *B. cereus* increased from 1 to 106.

Concerning, enterobacteriaceae, it is evident from Table 4 & Figure 1 that 42% of the examined ice cream mix powder samples were contaminated with these organisms. The range was from <100/g to $2x10^2$/g. A lower occurrence was recorded in ice cream mix powder with vanilla flavour (36%).

The results illustrated in Table 5 & Figure 1, reveal that total *coliforms*, *fecal coliforms* and *E. coli* were detected in 9, 2 and 0 % of the examined ice cream mix powder samples, respectively. The average counts of these microorganisms in the examined samples were 5, 4 and 0/g, respectively. A higher incidence of *coliforms* (16 %) was observed in the samples of dried ice cream mix with vanilla, while such organisms failed detection in the samples with chocolate. On the other hand, *fecal coliforms* were detected in one (4 %) sample from each of ice cream mix powder with mango and strawberry, but it could not be detected in the examined samples with chocolate and vanilla flavours. Higher findings of *coliforms* were reported by El-Bassiony and Aboul-Khier (1983). Also, the same authors isolated E. coli from these products. *Coliform and fecal coliform* bacteria can reach to the food processing plant and food service establishment from their original fecal, soil, water or plant environment. Therefore, these organisms are particularly useful as part of microbiological criteria to indicate post processing contamination.

Results presented in Table 6 and Figure 1 show that 23% of the examined samples of ice cream mix powder contained *enterococci* organisms with an average count of $2x10^3$/g. Higher incidence of *enterococci* was found in dried ice cream mix with strawberry flavour (36 %). Whereas, higher average value ($8x10^3$ bacteria/g) was counted in ice cream mix powder with chocolate flavour. El-Bassiony and Aboul-Khier (1983) obtained higher occurrences and lower counts of enterococci than the present results. The variation in results may be due to the difference in manufacturing practices, handling from producers to consumers and the effectiveness of hygienic measures applied during making of ice cream mix powder, packaging and storage. *Enterococci* microorganisms may have a distinctive role as indicator of poor factory sanitation, owing to their relatively high resistance to drying. Moreover, the public health significance can not be denied, specially when these organisms existed in tremendous numbers in the product as they have been implicated in several food poisoning outbreaks (Getting, et al., 1944; Deibel and Silliker, 1963 and ICMSF, 1978).

Regarding, the results of *staphylococci* and *micrococci* (Table 7 & Figure 1), no *Staph. aureus* was detected in all of the examined samples. *Staph. epidermidis* and *micrococci* were found in percentages of 21 and 8 % of examined samples, respectively. These results agree with those recorded by El-Bassiony and Aboul-Khier (1983). The presence of

staphylococci and micrococci in ice cream mix powder samples may indicate lack of personal hygiene.

As recorded in Table 8 & Figure 1, the *anaerobic sporeforming bacteria* were existed in 41 % of the examined samples. *Clostridia* organisms were presented in the examined samples in the following descending percentages: vanilla (56 %), chocolate (44 %), mango (36 %) and strawberry (28 %). The occurrence of *anaerobes* in the examined samples agrees to some extent with those recorded by El-Bassiony and Aboul-Khier (1983). Presence of *anaerobes* in the examined samples of ice cream mix powder could be used as an index of fecal or soil contamination of such products, and it was shown that there was a definite correlation between the hygienic condition of production and the content of *anaerobic sporeforming bacteria* in these products.

Table 9 and Figure 1 reveal that *yeasts and moulds* were found in all of the examined ice cream mix powder samples with a minimum of 1x10, a maximum of $9x10^3$ and an average count of $9x10^2$ *yeast & mould*/g. These results are parallel to those obtained by Aboul-Khier, et al. (1985). The high incidences and counts of yeast and mould in the examined samples of dried ice cream mix are indicative of the neglected sanitary measures adopted during processing, handling and packaging of these products. Also, the presence of *fungi* could constitute a public health hazard or may be responsible for undesirable changes and inferior quality of such products.

Unfortunately, ice cream mix powder, in spite of their low moisture content, may, at times, be responsible for transmitting some pathogenic microorganisms to consumers. These microorganisms may find opportunity, in absence of pasteurization, to multiply during preparation of ice cream, either at home or in small factories (small scale), to numbers sufficient to be of public health hazard. Furthermore, the objectionable heavy contamination of the dried products may be responsible for undesirable changes and inferior quality of dried ice cream mix used for preparation of ice cream. Therefore, strict hygienic measures and suitable regulations should be performed to dairy processing plants, methods of processing, packaging, storage and distribution of the dried milk products.

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Table 1. Aerobic plate counts of the examined ice cream mix powder samples.

| Ice cream mix powder | No. of examined samples | Positive samples | | Counts/g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Average |
| Chocolate flavoured | 25 | 25 | 100 | $1x10^3$ | $7x10^7$ | $7x10^6$ |
| Mango flavoured | 25 | 25 | 100 | $5x10^3$ | $8x10^7$ | $2x10^7$ |
| Strawberry flavoured | 25 | 25 | 100 | $1x10^3$ | $1x10^8$ | $2x10^7$ |
| Vanilla flavoured | 25 | 25 | 100 | $1x10^3$ | $8x10^7$ | $6x10^6$ |
| Total | 100 | 100 | 100 | $1x10^3$ | $1x10^8$ | $1x10^7$ |

Table 2. Psychrotrophic counts of the examined ice cream mix powder samples.

| Ice cream mix powder | No. of examined samples | Positive samples | | Counts/g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Average |
| Chocolate flavoured | 25 | 25 | 100 | $3x10^2$ | $3x10^5$ | $3x10^4$ |
| Mango flavoured | 25 | 25 | 100 | $2x10^2$ | $4x10^5$ | $6x10^4$ |
| Strawberry flavoured | 25 | 25 | 100 | $3x10^2$ | $7x10^5$ | $1x10^5$ |
| Vanilla flavoured | 25 | 25 | 100 | $2x10^2$ | $4x10^5$ | $7x10^4$ |
| Total | 100 | 100 | 100 | $2x10^2$ | $7x10^5$ | $8x10^4$ |

Table 3. Incidence and numbers of Bacillus cereus in the examined samples of ice cream mix powder.

| Ice cream mix powder | No. of examined samples | Positive samples | | Counts/g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Average |
| Chocolate flavoured | 25 | 20 | 80 | $2x10^2$ | $7x10^5$ | $8x10^4$ |
| Mango flavoured | 25 | 23 | 92 | <100 | $6x10^4$ | $1x10^4$ |
| Strawberry flavoured | 25 | 17 | 68 | $2x10^2$ | $2x10^4$ | $6x10^3$ |
| Vanilla flavoured | 25 | 19 | 76 | <100 | $3x10^5$ | $3x10^4$ |
| Total | 100 | 79 | 79 | <100 | $7x10^5$ | $3x10^4$ |

Table 4. Enterobacteriaceae counts of the examined ice cream mix powder samples.

| Ice cream mix powder | No. of examined samples | Positive samples | | Counts/g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Average |
| Chocolate flavoured | 25 | 11 | 44 | <100 | $1x10^2$ | 6x10 |
| Mango flavoured | 25 | 10 | 40 | <100 | $1x10^2$ | 4x10 |
| Strawberry flavoured | 25 | 12 | 48 | <100 | $2x10^2$ | 6x10 |
| Vanilla flavoured | 25 | 9 | 36 | <100 | $1x10^2$ | 4x10 |
| Total | 100 | 42 | 42 | <100 | $2x10^2$ | 5x10 |

Table 6. Enterococci counts of the examined ice cream mix powder samples.

| Ice cream mix powder | No. of examined samples | Positive samples | | Counts/g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Average |
| Chocolate flavoured | 25 | 4 | 16 | <100 | $1x10^4$ | $8x10^3$ |
| Mango flavoured | 25 | 3 | 12 | <100 | $1x10^3$ | $6x10^2$ |
| Strawberry flavoured | 25 | 9 | 36 | <100 | $1x10^3$ | $3x10^2$ |
| Vanilla flavoured | 25 | 7 | 28 | <100 | $6x10^3$ | $2x10^3$ |
| Total | 100 | 23 | 23 | <100 | $1x10^4$ | $2x10^3$ |

Table 7. Incidence of staphylococci and micrococci in the examined samples of ice cream mix powder.

| Ice cream mix powder | No. of examined samples | Staph. aureus | | Staph. epidermidis | | Micrococci | |
|---|---|---|---|---|---|---|---|
| | | Positive samples | | | | | |
| | | No. | % | No. | % | No. | % |
| Chocolate flavoured | 25 | 0 | 0 | 5 | 20 | 0 | 0 |
| Mango flavoured | 25 | 0 | 0 | 8 | 32 | 3 | 12 |
| Strawberry flavoured | 25 | 0 | 0 | 2 | 8 | 1 | 4 |
| Vanilla flavoured | 25 | 0 | 0 | 6 | 24 | 4 | 16 |
| Total | 100 | 0 | 0 | 21 | 21 | 8 | 8 |

Table 5. Incidence and counts of total coliforms and fecal coliforms in the examined samples of ice cream mix powder.

| Ice cream mix powder | No. of examined samples | Total coliforms | | | | | Fecal coliforms | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive samples | | Counts/g | | | Positive samples | | Counts/g | | |
| | | No. | % | Min. | Max. | Average | No. | % | Min. | Max. | Average |
| Chocolate flavoured | 25 | 0 | 0 | <3 | <3 | <3 | 0 | 0 | <3 | <3 | <3 |
| Mango flavoured | 25 | 3 | 12 | 3 | 9 | 5 | 1 | 4 | 3 | 3 | 3 |
| Strawberry flavoured | 25 | 2 | 8 | 4 | 7 | 6 | 1 | 4 | 4 | 4 | 4 |
| Vanilla flavoured | 25 | 4 | 16 | 3 | 7 | 4 | 0 | 0 | <3 | <3 | <3 |
| Total | 100 | 9 | 9 | <3 | 9 | 5 | 2 | 2 | <3 | 4 | 4 |

Table 8. Incidence of anaerobes in the examined samples of ice cream mix powder.

| Ice cream mix powder | No. of examined samples | Positive samples | |
|---|---|---|---|
| | | No. | % |
| Chocolate flavoured | 25 | 11 | 44 |
| Mango flavoured | 25 | 9 | 36 |
| Strawberry flavoured | 25 | 7 | 28 |
| Vanilla flavoured | 25 | 14 | 56 |
| Total | 100 | 41 | 41 |

Table 9. Total yeast and mould counts of the examined ice cream mix powder samples.

| Ice cream mix powder | No. of examined samples | Positive samples | | Counts/g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Average |
| Chocolate flavoured | 25 | 25 | 100 | 3x10 | $9x10^3$ | $1x10^3$ |
| Mango flavoured | 25 | 25 | 100 | 5x10 | $1x10^3$ | $3x10^2$ |
| Strawberry flavoured | 25 | 25 | 100 | 2x10 | $6x10^3$ | $9x10^2$ |
| Vanilla flavoured | 25 | 25 | 100 | 1x10 | $4x10^3$ | $8x10^2$ |
| Total | 100 | 100 | 100 | 1x10 | $9x10^3$ | $9x10^2$ |

**Fig. 1. Incidence of different microorganisms in the examined ice cream mix powder samples.**

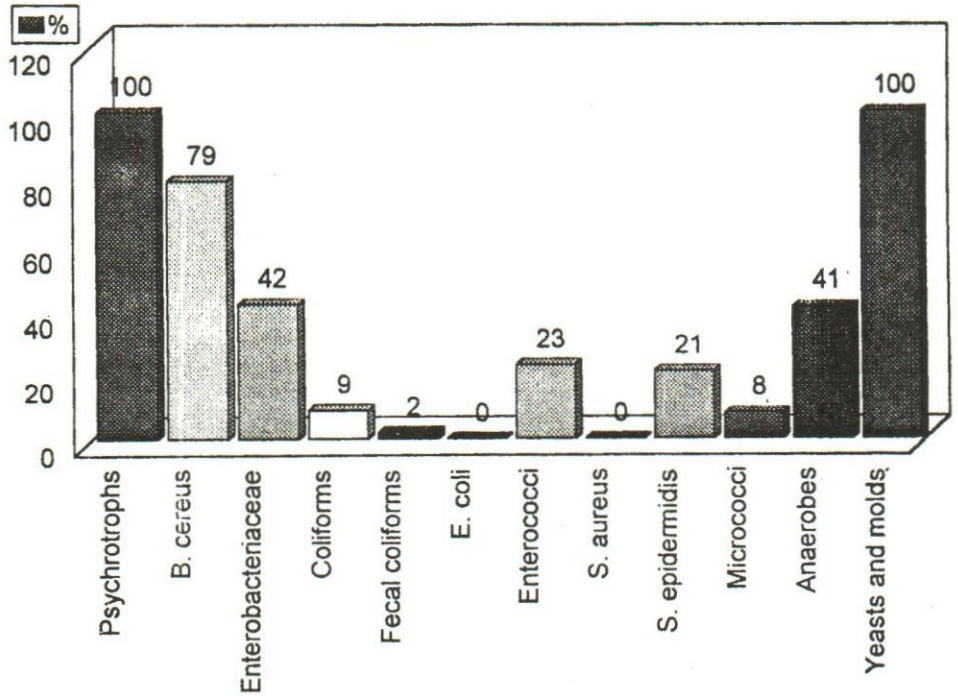


**Fig. 2. Frequency distribution of isolates in the examined ice cream mix powder samples.**